IS 12572-14 (1994): Biological Evaluation of Medical Devices, Part 14: Selection of Tests for Interactions with Blood [MHD 12: Hospital Equipment]

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भारतीय मानक

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Indian Standard

BIOLOGICAL EVALUATION OF MEDICAL DEVICES

PART 14 SELECTION OF TESTS FOR INTERACTIONS WITH BLOOD

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NATIONAL FOREWORD

This Indian Standard (Part 14) which is identical with ISO 10993-4 : 1992 'Biological evaluation of medical devices — Part 4 : Selection of tests for interactions with blood', issued by the International Organization for Standardization (ISO), was adopted by the Bureau of Indian Standards, on the recommendation of the Medical Instruments and Disposables Sectional Committee and approval of the Medical Equipment and Hospital Planning Division Council.

The selection and evaluation of any material or device intended for use in humans requires a structured programme of assessment. To give assurance that the final product will perform as intended and be safe for human use, the programme should include a biological evaluation, which should be planned and carried out by knowledgeable and experienced individuals capable of making informed decisions based on the advantages and disadvantages of various materials and test procedures available. This standard lays down the principles and guidelines governing the biological evaluation of interactions of medical devices with blood.

The text of the ISO standard has been approved as suitable for publication as Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

In this standard, the following International Standards are referred to. Read in their respective place, the following:

International Standard	*Indian Standard*	*Degree of Correspondence*
ISO 10993-1 : 1992 Biological evaluation of medical devices — Part 1 : Guidance on selection of tests	IS 12572 (Part 1) : 1994 Biological evaluation of medical devices: Part 1 Guidance on selection of tests (*first revision*)	Identical

Annexes A, B and C of this standard are for information only.

IS 12572 consists of the following parts, under the general title *'Guide for evaluation of medical devices for biological hazards':*

Part 1 Guidance on selection of tests (*first revision*)

Part 2 Animal welfare requirements

Part 3 Method of testing by tissue implantation

Part 4 Method of test for systemic toxicity : Assessment of acute toxicity of extracts from medical devices

Part 5 Method of test for intracutaneous reactivity of extracts from medical devices

Part 6 Method of test for systemic toxicity : Assessment of pyrogenicity in rabbits of extracts from medical devices

Part 7 Methods of test for sensitization : Assessment of potential of medical devices to produce delayed contact dermatitis

Part 8 Methods of test for skin irritation of extracts from medical devices

Part 9 Method of test for skin irritation by solid medical devices

(Continued on third cover)

Indian Standard

BIOLOGICAL EVALUATION OF MEDICAL DEVICES

PART 14 SELECTION OF TESTS FOR INTERACTIONS WITH BLOOD

1 Scope

This part of ISO 10993 gives guidance to agencies, manufacturers, research laboratories and others for evaluating the interactions of medical devices with blood.

It describes:

a) a classification of medical and dental devices that are intended for use in contact with blood, based on the intended use and duration of contact as defined in ISO 10993-1;

b) the fundamental principles governing the evaluation of the interaction of devices with blood;

c) the rationale for structured selection of tests, together with the principles and scientific basis of these tests.

Detailed requirements for testing cannot be specified because of the limitations in knowledge and precision of tests for interactions of devices with blood.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 10993. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10993-1:1992, *Biological evaluation of medical devices — Part 1: Guidance on selection of tests.*

3 Definitions

For the purposes of this part of ISO 10993, the definitions given in ISO 10993-1 and the following definitions apply.

3.1 blood/device interaction: Any interaction between blood or any component of blood and a device resulting in effects on the blood, or on any organ or tissue or on the device. Such effects may or may not have clinically significant or undesirable consequences.

3.2 *ex vivo*: Term applied to test systems that shunt blood directly from a human subject or test animal into a test chamber. If using an animal model, the blood may be shunted directly back into the animal (recirculating) or collected into test tubes for evaluation (single pass). In either case, the test chamber is located outside the body.

4 Abbreviations

Table 1 provides a list of abbreviations used in the context of this part of ISO 10993.

Table 1 — Abbreviations

Abbreviation	Meaning
Bb	Product of alternate pathway complement activation
β-TG	Beta-thromboglobulin
C4d	Product of classical pathway complement activation
C3a, C5a	(active) complement split products from C3 and C5
D-Dimer	Specific fibrin degradation products (F XIII cross-linked fibrin)
ECMO	Extracorporeal membrane oxygenator
E.M.	Electron microscopy
FDP	Fibrin/fibrinogen degradation products
FPA	Fibrinopeptide A
F_{1+2}	Prothrombin activation fragment 1 + 2
iC3b	Product of central C complement activation
IL-1	Interleukin-1
IVC	Inferior vena cava
MRI	Magnetic resonance imaging
PAC-1	Monoclonal antibody which recognizes the activated form of platelet surface glycoprotein IIb/IIIa
PET	Positron emission tomography
PF-4	Platelet factor 4
PT	Prothrombin time
PTT	Partial thromboplastin time
RIA	Radioimmunoassay
S-12	Monoclonal antibody which recognizes the alpha granule membrane component GMP140 exposed during the platelet release reaction
SC5b-9	Product of terminal pathway complement activation
TAT	Thrombin-antithrombin complex
TCC	Terminal complement complex
TT	Thrombin time
VWF	von Willebrand factor

5 Devices contacting blood

Devices contacting blood are categorized in ISO 10993-1:1992, clause 5.

5.1 Non-contact devices

(See subclause 5.1.1 of ISO 10993-1:1992.)

An example is *in vitro* diagnostic devices.

5.2 External communicating devices

(See subclause 5.1.3 of ISO 10993-1:1992.)

These are devices that contact the circulating blood and serve as a conduit into the vascular system. Examples include but are not limited to those in 5.2.1 and 5.2.2.

5.2.1 External communicating devices that serve as an indirect blood path [see subclause 5.1.3 a) of ISO 10993-1:1992] include but are not limited to

cannulae,

extension sets,

devices for the collection of blood,

devices for the storage and administration of blood and blood products (e.g. tubing, needles and bags).

5.2.2 External communicating devices in contact with circulating blood [see 5.1.3 c) of ISO 10993-1:1992] include but are not limited to

cardiopulmonary bypass,

extracorporeal membrane oxygenators,

haemodialysis equipment,

donor and therapeutic apheresis equipment,

devices for absorption of specific substances from blood,

interventional cardiology and vascular devices,

percutaneous circulatory support systems,

temporary pacemaker electrodes.

5.3 Implant devices

These are devices (see 5.1.4 of ISO 10993-1:1992) that are placed largely or entirely within the vascular system. Examples include but are not limited to

mechanical or tissue heart valves,

prosthetic or tissue vascular grafts,

circulatory support devices (ventricular-assist devices, artificial hearts, intra-aortic balloon pumps),

inferior vena cava filters,

stents,

arteriovenous shunts,

blood monitors,

internal drug delivery catheters,

pacemaker electrodes,

intravascular membrane oxygenators (artificial lungs).

6 Tests

6.1 General recommendations

6.1.1 Where possible, tests should use an appropriate model or system which simulates the geometry and conditions of contact of the device with blood during clinical applications, including duration of contact, temperature, sterile condition and flow conditions. For devices of defined geometry such as vascular grafts of varying lengths, the relation of surface area (length) to test results should be evaluated.

The selected methods and parameters should be in accordance with the current state of the art.

NOTE 1 Only blood-contacting parts should be tested.

6.1.2 Controls shall be used unless their omission can be justified. Where possible, testing should include a device already in clinical use or well-characterized reference materials. Several materials and configurations are available (see ISO 10993-12 [7]).

Reference materials used should include negative and positive controls. All materials tested should meet all quality control and quality assurance procedures of the manufacturer and test laboratory and should be identified as to source, manufacturer, grade and type.

6.1.3 Testing of materials which are candidates to be components of a device should be conducted for screening purposes. However, such tests do not serve as a substitute for the requirement that the complete device be tested under conditions which simulate clinical application.

6.1.4 Tests which do not simulate the conditions of a device during use may not predict accurately the nature of the blood/device interactions which may occur during clinical applications. For example, some short-term *in vitro* or *ex vivo* tests are poor predictors of long-term *in vivo* blood/device interactions [22], [23].

6.1.5 It follows from the above that devices whose intended use is *ex vivo* (external communicating) should be tested *ex vivo* and devices whose intended use is *in vivo* (implants) should be tested *in vivo* in an animal model under conditions simulating where possible clinical use.

6.1.6 *In vitro* tests are regarded as useful in screening external communicating devices or implants but may not be accurate predictors of blood/device interactions occurring upon prolonged or repeated exposure or permanent contact (see 6.3.2). Devices intended for non-contact use only do not require evaluation of blood/device interactions. Devices which come into very brief contact with circulating blood (e.g. lancets, hypodermic needles, capillary tubes) generally do not require blood/device interaction testing.

6.1.7 The two recommendations in 6.1.5 and 6.1.6, together with clause 5, serve as a guide for the selection of tests listed in 6.2.1.

6.1.8 Disposable laboratory equipment used for the collection of blood and performance of *in vitro* tests on blood should be validated to ascertain that there is no significant interference with the test being performed. This can be conducted by performing tests on reference standards and comparing results with those obtained by a clinically approved technique.

6.1.9 If tests are selected in the manner described and testing is conducted under conditions which simulate clinical applications, the results of such testing have the greatest probability of predicting clinical performance of devices. However, species differences and other factors may limit the predictability of any test.

6.1.10 Because of species differences in blood reactivity, human blood should be used where possible. When animal models are necessary, for example for evaluation of devices used for prolonged or repeated exposure or permanent contact, species differences in blood reactivity should be considered. Blood values and reactivity between humans and non-human primates are very similar [23].

NOTE 2 The use of non-human primates for blood compatibility and medical device testing is prohibited by EC law (86/906/EEC) and some national laws.

However, the use of species other than non-human primates such as the pig, calf or sheep may also yield satisfactory results. The canine model has been found useful in the pre-clinical evaluation of prosthetic vascular grafts [43]. Because species differences may be significant (for example platelet adhesion, thrombosis, [17] and haemolysis tends to occur more readily in the canine species than in the human), all results of animal studies should be interpreted with caution.

6.1.11 The use of anticoagulants should be avoided unless the device is designed to perform in their presence. The choice and concentration of anticoagulant used influence blood/device interactions. Devices that are used with anticoagulants shall be assessed using anticoagulants in the range of concentrations used clinically.

6.1.12 Minor modifications in a clinically accepted device may cause significant changes in its clinical functions. Examples of such modifications include changes in design, changes in surface or bulk chemical composition of materials and changes in texture, porosity or other properties of vascular grafts. Therefore the effect of such changes on blood/device interactions shall be considered for clinical significance.

6.1.13 A sufficient number of tests including suitable controls shall be performed to permit statistical evaluation of the data. The variability in some test methods requires that those tests be repeated a sufficient number of times to determine significance. In addition repeated studies over an extended period of blood/device contact provide information about the time-dependence of the interactions. A statistician should be consulted in the early stages of experimental design.

6.2 Test methods

6.2.1 Recommended tests for interactions of devices and materials with blood

Recommended tests are organized on the basis of the type of device:

Table 2: External communicating devices — Level 1 — blood path, indirect

Table 3: External communicating devices — Level 1 — circulating blood

Table 4: External communicating devices — Level 2 — optional

Table 5: Implant devices — Level 1

Table 6: Implant devices — Level 2 — optional

Level 1 and level 2 tests are classified into five categories based on the primary process or system being measured:

a) thrombosis,

b) coagulation,

c) platelets and platelet functions,

d) haematology,

e) immunology.

Two levels of tests are presented. Select one or more test method(s) from each category of level 1 tests (tables 2, 3 and 5), in order to obtain the maximum information about the spectrum of reactions initiated when a device contacts blood.

Additional testing from level 2 (tables 4 and 6) is optional. The principles and scientific basis for these tests are presented in annex B.

6.2.1.1 Non-contact devices

These devices generally do not require blood/device interaction testing. Disposable test kits should be validated to rule out interference of materials with test accuracy.

6.2.1.2 External communicating devices

The external communicating devices and their test methods are listed in tables 2 to 4. These test methods are recommended for devices intended for limited (LI, < 24 h) and prolonged or repeated (PR, 24 h to 30 days) exposure. See also 6.1.6.

6.2.1.3 Implant devices

The implant devices and their test methods are listed in tables 5 and 6. These test methods are recommended for devices intended for prolonged or repeated (PR, 24 h to 30 days) exposure or permanent contact (PC, > 30 days).

6.2.2 Indications and limitations

Table 7 gives a list of commercially available assays validated for use with human blood and tables 2 to 6 present a list of tests. Level 1 tests, which shall be considered, are relatively simple for general use in evaluating the interactions of materials and devices with blood. Level 2 tests are more sophisticated, require special expertise in performance and interpretation, and are regarded as optional. In both categories, strict attention to technical detail is required. RIAs are available for human blood testing but are not generally

available for other species. The human test kits usually do not cross-react with other species except for some non-human primates. Care should be taken when designing test systems to ensure that one is actually measuring activation due to the test material and not an artifact of the system.

Discrepancies in evaluating blood/device interactions may occur because of inadequate materials characterization or inappropriate handling before blood tests are performed. For example the studies may have relied on only one type of test or may have permitted the introduction of foreign material unrelated to the material or device under test. Materials to be used in a low flow (venous) environment may interact with blood quite differently when used in high flow (arterial) situations. Changes in design and/or flow conditions can alter the apparent *in vivo* haemocompatibility of a material.

6.3 Types of tests

6.3.1 *In vitro* tests

Variables that should be considered when using *in vitro* test methods include haematocrit, anticoagulants, sample collection, sample age, aeration and pH, temperature, sequence of test versus control studies, surface-to-volume ratio, and fluid dynamic conditions (especially wall shear rate). Tests should be performed with minimal delay, usually within 4 h, since some properties of blood change rapidly following collection.

6.3.1.1 Platelet tests

Blood collection techniques should be reproducible. Platelets can become hyperreactive under a variety of conditions, including improper blood collection. Tests to verify normal platelet reactivity are usually performed with an aggregometer. Platelet preparations with reduced reactivity are easily detected using this method, but hyperreactive platelets are not normally detected. Platelet aggregation tests can be modified (by appropriately reducing the concentration of platelets or aggregating agents) to determine if platelets become hyperreactive following exposure to a material or device.

6.3.1.2 Coagulation tests

Coagulation methods are based on the use of native (fresh, non-anticoagulated) whole blood, anticoagulated whole blood (usually citrated), platelet-rich plasma or platelet-poor plasma. Since most of the standard coagulation assays are designed to detect clinical coagulation disorders which result in delayed clotting or excessive bleeding, the protocols for evaluating blood/device interactions should be modified appropriately to evaluate accelerated coagulation induced by biomaterials. Reagents for tests based on the activated partial thromboplastin time include an activator such as kaolin, celite, or ellagic acid. Reagents with such activators should be avoided because they tend to mask the acceleration of coagulation which materials and devices cause. The material to be tested serves as the activator; controls (without the material) should be included.

Blood is exposed to test materials either in a static chamber such as a parallel plate cell or in a closed-loop system where the inner surface of the tubing is the test material. After a predetermined contact time with the test surface, tests of the surface and blood can be conducted.

Table 2 — External communicating devices — Level 1 — Blood path, indirect (see 5.2.1)

Test category	Method	Comments
Thrombosis	Light microscopy (adhered platelets, leucocytes, aggregates, erythrocytes, fibrin, etc.)	Light microscopy can be replaced by scanning E.M. if the nature of the material presents technical problems for light microscopy.
Coagulation	PTT (non-activated)	
Platelets	Platelet count	
Haematology	Leucocyte count and differential; haemolysis (plasma haemoglobin)	Haemolysis is regarded as an especially significant screening test to perform in this category because of its measurement of red blood cell membrane fragility in contact with materials and devices. The method used should be one of the normative standard test methods for haemolysis.
Immunology	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9	A panel including the last four tests encompasses the various complement activation pathways.

Table 3 — External communicating devices — Level 1 — Circulating blood (see 5.2.2)

Test category	Method	Comments
Thrombosis	Per cent occlusion; flow reduction; gravimetric analysis (thrombus mass); light microscopy (adhered platelets, leucocytes, aggregates, erythrocytes, fibrin, etc.); pressure drop across device	Light microscopy can be replaced by scanning E.M. if the nature of the material presents technical problems for light microscopy. Pressure drop not recommended for devices intended for PR (see 6.2.1.2).
Coagulation	PTT (non-activated)	
Platelets	Platelet count; platelet aggregation; template bleeding time	
Haematology	Leucocyte count and differential; haemolysis (plasma haemoglobin)	Haemolysis is regarded as an especially significant screening test to perform in this category because of its measurement of red blood cell membrane fragility in contact with materials and devices. The method used should be one of the normative standard test methods for haemolysis.
Immunology	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9	A panel including the last four tests encompass the various complement activation pathways.

Table 4 — External communicating devices — Level 2 — *optional*

Test category	Method	Comments
Thrombosis	Scanning E.M. (platelet adhesion and aggregation; platelet and leucocyte morphology; fibrin)	
Coagulation	Specific coagulation factor assays; FPA, D-dimer, F_{1+2}, PAC-1, S-12, TAT	
Platelets	PF-4, β-TG; thromboxane B2; gamma imaging of radiolabelled platelets ^{111}In-labelled platelet survival	^{111}In-labelling is recommended for PR only (see 6.2.1.2).
Haematology	Reticulocyte count; activation specific release products of peripheral blood cells (i.e. granulocytes)	Recommended for PR only (see 6.2.1.2).
Immunology	IL-1 and other cytokines; detection of messenger-RNA specific for cytokines	

Table 5 — Implant devices — Level 1 (see 6.3)

Test category	Method	Comments
Thrombosis	Per cent occlusion; flow reduction; autopsy of device (gross and microscopic); autopsy of distal organs (gross and microscopic)	
Coagulation	PTT (non-activated), PT, TT; plasma fibrinogen, FDP	
Platelets	Platelet count; platelet aggregation	
Haematology	Leucocyte count and differential; haemolysis (plasma haemoglobin)	Haemolysis is regarded as an especially significant screening test to perform in this category because of its measurement of red blood cell membrane fragility in contact with materials and devices. The method used should be one of the normative standard test methods for haemolysis.
Immunology	C3a, C5a, TCC, Bb, iC3b, C4b, SC5b-9	A panel including the last four tests encompassses the various complement activation pathways.

Table 6 — Implant devices — Level 2 — optional

Test category	Method	Comments
Thrombosis	Scannning E.M.; angiography	
Coagulation	Specific coagulation factor assays; FPA, D-dimer, F_{1+2}, PAC-1, S-12, TAT	
Platelets	^{111}In-labelled platelet survival PF-4, β-TG thromboxane B2; gamma imaging of radiolabelled platelets	
Haematology	Reticulocyte count; activation specific release products of peripheral blood cells (i.e. granulocytes)	
Immunology	IL-1 and other cytokines; detection of messenger-RNA specific for cytokines	

6.3.2 *Ex vivo* tests

Ex vivo tests should be performed when the intended use of the device is *ex vivo*, for example an external communicating device. *Ex vivo* testing may also be useful when the intended use is *in vivo*, for example an implant such as a vascular graft. Such use should not however substitute for an implant test.

Ex vivo test systems are available for monitoring platelet adhesion, emboli generation, fibrinogen deposition, thrombus mass, white cell adhesion, platelet consumption, and platelet activation [17], [27], [43]. Blood flow-rates can be measured with either Doppler or electromagnetic flow probes. Alterations in flow-rates may indicate the extent and course of thrombus deposition and embolization.

Many *ex vivo* test systems use radiolabelled blood components to monitor blood/device interactions. Radiolabelled platelets and fibrinogen are the most commonly labelled components of blood. Alteration of platelet reactivity by the labelling procedure can be minimized by strict attention to technical detail [20], [21], [32].

Advantages of *ex vivo* tests over *in vitro* tests are that flowing native blood is used (thus eliminating artifacts caused by anticoagulants as well as providing physiological flow conditions), several materials can be evaluated since the chambers can be changed, and it is possible to monitor some events in real time. Some disadvantages include variability in blood flow from one experiment to another, variable blood reactivity from one animal to the other, and the usually relatively short time intervals that can be evaluated. Positive and negative controls using the same animal are recommended in this regard.

6.3.3 *In vivo* tests

In vivo testing involves implanting the material or device in animals. Vascular patches, vascular grafts, prosthetic rings, heart valves and circulatory-assist devices are examples of configurations used in *in vivo* testing. *In vivo* tests are usually designed to examine haemocompatibility over a period longer than 24 h.

Patency (of a conduit) is the most common measure of success or failure for most *in vivo* experiments. The per cent occlusion and thrombus mass are determined after the device is removed. The tendency of thrombi formed on a device to embolize to distal organs should be assessed by a careful gross as well as microscopic examination of organs downstream from the device. The kidneys are especially prone to trap thrombi which have embolized from devices implanted upstream from the renal arteries (for example ventricular-assist devices, artificial hearts, aortic prosthetic grafts) [16].

Methods to evaluate *in vivo* performance without terminating the experiment are available. Arteriograms are used to determine graft patency or thrombus deposition on devices. Radioimaging can be used to monitor platelet deposition at various time periods *in vivo*; platelet survival and consumption can be used as indicators of blood/device interactions and passivation due to neointima formation or protein adsorption.

In some *in vivo* test systems the material's properties may not be major determinants of the blood/device interactions. Flow parameters, compliance, porosity and implant design may be more important than blood compatibility of the material itself. As an example, low flow-rate systems may give substantially different results when compared to the same material evaluated in a high flow-rate system. In such cases, test system performance *in vivo* should carry more importance than *in vitro* test results.

Table 7 — Commercially available assays for platelet, coagulation, fibrinolytic and complement factors

Factor	Types of assay
Plasminogen	Colorimetric Fluorogenic
Antithrombin III	Chromogenic, fluorogenic
Protein C	Chromogenic
Protein S	Chromogenic
Antiplasmin	Chromogenic
Prekallikrein	Chromogenic
Kallikrein	Fluorogenic
PF-4	RIA, ELISA
	Chromogenic
β-TG	RIA, ELISA
Thromboxane B2	RIA, ELISA
Factor VIII-VWF	Chromogenic; clotting time
Factor IX	Chromogenic; clotting time
Factor IXa	Fluorogenic
Factor X	Chromogenic; clotting time
Factor Xa	Fluorogenic
Factor XII	Chromogenic; clotting time
Factor XIIa	Fluorogenic
FPA	RIA, ELISA
$C3_a$, $C5_a$	RIA
Bb, iC3b, C4d, SC5b-9	ELISA
TCC	ELISA
TAT	RIA, ELISA
IL-1	RIA, ELISA
F_{1+2}	RIA, ELISA
D-dimer	RIA, ELISA

NOTE — These assays have been validated for human use. Validation of their accuracy for other species shall be determined prior to use.

Annex A
(informative)

Evaluation of cardiovascular devices and prostheses during *in vivo* function

A.1 General considerations

A.1.1 This annex provides background for selecting tests to evaluate the interactions of cardiovascular devices with blood. Subclause 6.2.1 contains a complete list of recommended (level 1) and optional (level 2) tests for evaluating blood/device interactions of the three categories of devices: non-contact, external communicating, and implant.

A.1.2 The following classification of blood/device interactions is provided as background.

A.1.2.1 Interactions which mainly affect the device and which may or may not have an undesirable effect on the subject are as follows:

a) adsorption of plasma proteins, lipids, calcium or other substances from the blood onto the surface of the device; or absorption of such substances into the device;

b) adhesion of platelets, leucocytes or erythrocytes onto the surface of the device, or absorption of their components into the device;

c) formation of pseudointima or tissue capsule on the surface of the device;

d) alterations in mechanical and other properties of the device.

A.1.2.2 Interactions which have a potentially undesirable effect on the animal or human are as follows:

a) activation of platelets, leucocytes or other cells, or activation of the coagulation, fibrinolytic, complement, or other pathways, including immunotoxicity (immunosuppression, immunopotentiation, immunomodulation);

b) formation of thrombi on the device surface;

c) embolization of thrombotic or other material from the device's luminal surface to another site within the circulation;

d) injury to circulating blood cells resulting in anaemia, haemolysis, leucopenia, thrombocytopenia or altered function of blood cells;

e) injury to cells and tissues adjacent to the device;

f) intimal hyperplasia or accumulation of other tissue on or adjacent to the device, resulting in reduced flow or affecting other functions of the device;

g) adhesion and growth of bacteria or other infectious agents to or near the device.

A.1.3 Procedures used to evaluate cardiovascular devices in animals are essentially the same as those employed in the clinical setting. However, animal models permit continuous device monitoring and systematic controlled study of important variables.

A.1.4 The test protocols recommended follow certain general guidelines. Thrombosis, thromboembolism, bleeding and infection are the major deterrents to the use and further development of advanced cardiovascular prostheses. For devices with limited blood exposure (< 24 h), important measurements are related to the acute extent of variation of haematologic, haemodynamic and performance variables, gross thrombus formation and possible embolism. With prolonged or repeated exposure, or permanent contact (> 24 h), emphasis is placed on serial measurement techniques that may yield information regarding the time course of thrombosis and thromboembolism, the consumption of circulating blood components, the development of intimal hyperplasia and infection. In both of the above exposure and contact categories, assessment of haemolysis is important. Thrombus formation may be greatly influenced by surgical technique, variable time-dependent thrombolytic and embolic phenomena, superimposed device infections and possible alterations in exposed surfaces, for example intimal hyperplasia and endothelialization.

The consequences of the interaction of artificial surfaces with the blood may range from gross thrombosis and embolization to subtle effects such as accelerated consumption of haemostatic elements; the latter may be compensated or lead to depletion of platelets or plasma coagulation factors.

A.1.5 Disturbances of organ function may occur due to blood/device interaction. For instance kidney function and pulmonary function may be affected by activated blood coagulation and platelet/leucocyte/complement interactions.

Platelet survival and plasma levels of the platelet-specific proteins PF-4 and β-TG may reflect the extent of platelet activation *in vivo* (and perhaps risk of thromboembolism) even in the absence of significantly elevated rates of platelet consumption. The template bleeding time is an index of *in vivo* platelet function; a prolonged value suggests thrombocytopenia or a qualitative platelet disorder, such as may occur during cardiopulmonary bypass [28]. Measurements of FPA may indicate activation of intrinsic coagulation.

Localization of thrombotic material by radionuclide imaging techniques using radiolabelled platelets has been demonstrated in studies of vascular grafts, valve prostheses, and other devices, both implants and externally communicating devices [20], [21], [36]. In addition, duplex scanning and a careful examination of the explanted device can provide very useful information [37], [38].

A.1.6 The choice of an animal model may be restricted by size requirements, the availability of certain species and cost. It is critical that the investigators be mindful of the physiological differences and similarities of the species chosen with those of the human, particularly those relating to coagulation, platelet functions and fibrinolysis, and the response to pharmacological agents such as anaesthetics, anticoagulants, thrombolytic and antiplatelet agents, and antibiotics. Because of species differences in reactivity and variable responses to different devices, data obtained from a single species should be interpreted with caution. Non-human primates such as baboons bear a close similarity in haematologic values, blood coagulation mechanism and cardiovascular system to the human [27]. An additional advantage of a non-human primate is that many of the immunologic probes for thrombosis developed for humans are suitable for use in other primates. These probes include PF-4, β-TG, FPA, TAT, and F_{1+2}. The dog is a commonly used species and has provided useful information; however, device-related thrombosis in the dog tends to occur more readily than in the human, a difference which can be viewed as an advantage when evaluating this complication. The pig is generally regarded as a suitable animal model because of its haematologic and cardiovascular similarities to the human. The effect of the surgical implant procedure on results should be kept in mind and appropriate controls included.

A.2 Cannulae

Cannulae are typically inserted into one or more major blood vessels to provide repeated blood access. They are also used during cardiopulmonary bypass and other procedures. They may be tested acutely or chronically and are commonly studied as arteriovenous (AV) shunts. The use of cannulae appears to induce little alteration in the levels of circulating blood cells or clotting factors. Cannulae, like other indirect blood path devices (5.2.1), generally require less testing than devices in circulating blood (6.2.1.2).

A.3 Catheters and guidewires

Most of the tests considered under cannulae are relevant to the study of catheters and guidewires. The location or placement of catheters in the arterial or venous system can have a major effect on blood/device interactions. It is advised that simultaneous control studies be performed using a contralateral artery or vein. Care should be taken not to strip off thrombus upon catheter withdrawal. *In situ* evaluation may permit assessment of the extent to which intimal or entrance site injuries contributed to the thrombotic process. Kinetic studies with radiolabelled blood constituents are recommended only with chronic catheters, but may be useful for imaging thrombus accumulation *in vivo*. Angiography and Doppler blood flow measurements may also be useful.

A.4 Extracorporeal oxygenators, haemodialyzers, therapeutic apheresis equipment, and devices for absorption of specific substances from blood

The haemostatic response to cardiopulmonary bypass may be significant and acute. Many variables such as use of blood suction, composition of blood pump priming fluid, hypothermia, blood contact with air and time of exposure influence test values. Emboli in outflow lines may be detected by the periodic placement of blood filters *ex vivo*, or the use of ultrasonic radiation or other non-invasive techniques. Thrombus accumulation can be indirectly assessed during bypass by monitoring performance factors such as pressure drop across the oxygenator and oxygen transfer rate. An acquired transient platelet dysfunction associated with selective alpha granule release has been observed in patients on cardiopulmonary bypass [28]; the template bleeding time and other tests of platelet function and release are particularly useful.

Complement activation is caused by both haemodialyzers and cardiopulmonary bypass apparatus. Clinically significant pulmonary leucostasis and lung injury with dysfunction may result. For these reasons, it is useful to quantify complement activation with these devices.

Therapeutic apheresis equipment and devices for absorption of specific substances from the blood, because of their high surface-to-volume ratio, can potentially activate complement, coagulation, platelet and leucocyte pathways. Examination of blood/device interactions should follow the same principles as for extracorporeal oxygenators and haemodialyzers.

A.5 Ventricular-assist devices

These devices may induce considerable alteration in various blood components. Factors contributing to such effects include the large foreign surface area to which blood is exposed, the high flow regimes and the regions of disturbed flow such as turbulence or separated flow. Tests of such devices may include measurements of haemolysis, platelet and fibrinogen concentration, platelet survival, complement activation, and close monitoring of liver, renal, pulmonary and central nervous system function. A detailed pathologic examination at surgical retrieval is an important component of the evaluation [37], [38].

A.6 Heart valve prostheses

Invasive, non-invasive and *in vitro* hydrodynamic studies are important in the assessment of prosthetic valves.

2D and M mode echocardiography makes use of ultrasonic radiation to form images of the heart. Reflections from materials with different acoustic impedances are received and processed to form an image. The structure of prosthetic valves can be examined. Mechanical prostheses emit strong echo signals and the movement of the occluder can usually be clearly imaged. However the quality of the image may depend upon the particular valve being examined. Echocardiography may also be useful in the assessment of function of tissue-derived valve prostheses. Vegetations, clots and evidence of thickening of the valve leaflets are elucidated. Using conventional and colour flow Doppler echocardiography, regurgitation can be identified and semi-quantified.

Measurements of platelet survival and aggregation, blood tests of thrombosis and haemolysis, pressure and flow measurements, and autopsy of the valve and adjacent tissues are also recommended.

A.7 Vascular grafts

Both porous and non-porous materials can be implanted at various locations in the arterial or venous system. The choice of implantation site is determined largely by the intended use for the prosthesis. Patency of a given graft is enhanced by larger diameter and shorter length. A rule of thumb for grafts less than 4 mm ID is that the length should exceed the diameter by a factor of 10 (i.e., 40 mm for a 4 mm graft) for a valid model. Patency can be documented by palpation of distal pulses in some locations and by periodic angiography. Ultrasonic radiation, MRI, and PET may also be useful. Results of serial radiolabelled platelet imaging studies correlate with the area of non-endothelialized graft surface in baboons [27]. Radiolabelled platelets facilitate non-invasive imaging of mural thrombotic accumulations. Serial measurements of platelet count, platelet release constituents, fibrinogen/fibrin degradation products and activated coagulation species also are recommended. Autopsy of the graft and adjacent vascular segments for morphometric studies of endothelial integrity and proliferative response can provide valuable information.

A.8 IVC filters and stents

These devices can be studied by angiography and ultrasonic radiation. Other techniques useful for vascular graft evaluation (see A.7) are appropriate here as well.

Annex B
(informative)

Laboratory tests: principles and scientific basis

B.1 General

B.1.1 The principles and scientific basis of the tests listed in 6.2.1 are described here. Detailed methods are found in standard texts of laboratory medicine and clinical pathology. References [14] to [39] and [41] to [44] describe tests which may be useful in the evaluation of blood/device interactions. Because of both biological variability and technical limitations, the accuracy of many of these tests is limited. When possible, the tests should be repeated a sufficient number of times to determine the significance of the results.

B.1.2 In order for tests to be of use in the *in vitro* evaluation of blood/device interactions, anticoagulated blood or plasma collected from normal human subjects or experimental animals should first be exposed to the material or device under standardized conditions including time, temperature and flow. An aliquot of the exposed blood or plasma is then tested shortly after exposure. Conditions of exposure should be based on the intended use of the device. One proposed set of conditions is as follows:

a) time of exposure: not less than 15 min;

b) temperature: 37 °C;

c) flow: to simulate intended use of the device;

d) start of test: within 15 min following exposure.

However, the appropriate conditions depend on the device or material being tested and its intended end-use.

B.1.3 When evaluating externally communicating devices and implant devices while in their in-use position, blood is collected into an anticoagulant and the test is performed as described without a prior exposure stage. The tests are classified into five categories, as defined in 6.2.1, according to the process or system being tested: thrombosis, coagulation, platelets and platelet functions, haematology and immunology.

B.2 Thrombosis

B.2.1 Per cent occlusion

Per cent occlusion is visually quantified after a device has been in use and has been removed. This is a measure of the severity of the thrombotic process in a conduit. Lack of occlusion does not necessarily eliminate the existence of a thrombotic process, since thrombi may have embolized or been dislodged before per cent occlusion is measured. Occlusion may be caused not only by thrombosis but also by intimal hyperplasia, especially at perianastomotic sites in vascular grafts; microscopic examination is required to identify the nature of the occlusive process.

B.2.2 Flow reduction

Flow (rate or volume) is measured after a period of use. Measurements may be performed either during use, or before and after use. Rationale and interpretation are the same as B.2.1.

B.2.3 Gravimetric analysis (thrombus mass)

This is conducted after removal of the device from the in-use position. Rationale and interpretation are as for B.2.1.

B.2.4 Light microscopy

By this technique, information can be obtained regarding the density of cells, cellular aggregates and fibrin adherent to materials, as well as the geographic distribution of these deposits on the materials or device. The method is semi-quantitative.

B.2.5 Pressure drop across device

This is measured before and after a period of use. Rationale and interpretation are as for B.2.1.

B.2.6 Scanning E.M.

Rationale and interpretation are the same as B.2.4. This method has the advantage over B.2.4 of providing greater detail about fine structure of components being examined. Quantitative conclusions require sufficient replicate determinations to establish degree of reproducibility.

B.2.7 Autopsy of device

This method is of great importance in evaluating the biological responses to implanted devices. The distribution, size and microscopic nature of cellular deposits can best be determined by a careful and detailed autopsy. Proposed procedures have been published [37], [38].

B.2.8 Autopsy of distal organs

The rationale is to examine for distal effects of implanted devices. These effects include thromboembolism, infection and embolization of components of the device.

B.2.9 Angiography

This method is used to determine patency or degree of narrowing of a graft or other conduit and to detect thrombus deposition on devices during their *in vivo* performance.

B.3 Coagulation

B.3.1 Partial Thromboplastin Time (PTT)

The partial thromboplastin time [35] is the clotting time of recalcified citrated plasma on the addition of partial thromboplastin. Partial thromboplastin is a phospholipid suspension usually extracted from tissue thromboplastin, the homogenate from mammalian brain or lung. Shortening of the PTT following contact with a material under standard conditions indicates activation of the contact phase of blood coagulation. A prolonged PTT suggests a deficiency in any of the plasma coagulation factors I (fibrinogen), II (prothrombin), V, VIII, IX, X, XI, or XII, but not VII or XIII. Heparin and other anticoagulants also cause a prolonged PTT.

Partial thromboplastin reagents using various activating substances such as kaolin or celite are commercially available. Using these reagents, the test is called the activated partial thromboplastin time (APTT). The APTT is of no value in the *in vitro* evaluation of blood/device interactions because the activating substances mask any activation caused by the device or its component materials.

B.3.2 Prothrombin Time (PT)

Blood is mixed with a measured amount of citrate and the plasma is obtained by centrifugation [35]. This test is based on having an optimum concentration of calcium ions and an excess of thromboplastin, the only variable being the concentration of prothrombin and accessory factors in a carefully measured volume of plasma. Oxalate is not recommended as the anticoagulant because factor V is less stable in this solution and the PT may become prolonged as a result.

This test measures prothrombin and accessory factors. In the presence of tissue thromboplastin, the clotting time depends on the concentrations of prothrombin, factor V, factor VII and factor X (assuming fibrinogen, fibrinolytic and anticoagulant activity to be normal). A prolonged prothrombin time generally indicates a deficiency of prothrombin or factor V, VII, X or fibrinogen. This test is useful for evaluating implant devices only.

B.3.3 Thrombin Time (TT)

The thrombin time [35] is the time required for plasma to clot when a solution of thrombin is added. The thrombin time is prolonged with a deficiency in fibrinogen (below 100 mg/dl), qualitative abnormalities in fibrinogen and elevated levels of FDP or heparin. The test is useful for evaluating implant devices only.

B.3.4 Fibrinogen

Dysfibrinogenemia, afibrinogenemia and hypofibrinogenemia cause prolonged PT, PTT and TT results [18]. The screening test most sensitive to fibrinogen deficiency is the TT. If the exact level of fibrinogen is needed, a commercially available modified TT is recommended. The test is useful for evaluating implant devices only.

B.3.5 Fibrinogen and fibrin degradation products (FDP)

There are several commercially available test kits for immunoassay of FDP [18]. Purified fragments D and E are administered to laboratory animals, which respond with production of specific immunoglobulins. The immunoglobulins are harvested, extracted, and purified to yield specific antisera at controlled concentrations. A suspension of latex particles is coated with the anti-FDP globulin standardized to be sensitive to FDPs at 2 µg/ml. It is important to bear in mind that such immunoassays may be of no value when used with the blood of some animal species.

Normal physiological fibrinolysis yields the FDPs X, Y, C, D and E in concentrations below 2 µg/ml of plasma. The normally low level of FDPs is maintained by the low rate of the degradation reaction and the high rate of clearance of FDPs from the circulation. Pathologic degradation of fibrin and fibrinogen, a result of increased plasminogen activation, yields FDP of 2 µg/ml to 40 µg/ml or more. The test is useful for evaluating implant devices only.

B.3.6 Specific coagulation factor assays

Significant reduction (e.g. to less than 50% of the normal or control level) of coagulation factors following exposure of blood to a material or device under standard conditions suggests accelerated consump-

tion of those factors by adsorption, coagulation or other mechanisms.

B.3.7 FPA, D-dimer, F_{1+2}, TAT

Elevated levels of FPA, D-dimer, or F_{1+2} indicate activation of the coagulation mechanism. Elevated TAT complexes indicate activation of blood coagulation and formation of a complex between thrombin being generated and circulating antithrombin.

B.4 Platelets and platelet functions

B.4.1 Platelet count

It is important to determine the platelet count [18], [44] because of the key role of platelets in preventing bleeding. A significant drop in platelet count of blood exposed to a device may be caused by platelet adhesion, platelet aggregation, platelet sequestration (for example in the spleen), or blood coagulation on materials or devices. A reduction in platelet count during use of an implanted device may also be caused by accelerated destruction or removal of platelets from the circulation.

B.4.1.1 Manual platelet count

Platelet counts are performed manually in some clinical laboratories despite the wide availability of highly accurate platelet counting instruments (B.4.1.2).

B.4.1.2 Automated platelet count using whole blood or platelet-rich plasma

Automated platelet counts may be performed using either well mixed whole blood or platelet-rich plasma (PRP). The use of whole blood is preferred because using PRP may yield inaccurate results. Instrument counts are made on a greater number of particles than manual counts. Consequently, the coefficient of variation for automated counts may be as small as 4 %, whereas the best achievable for the phase microscope manual count is 11 %. Most instruments also contain circuitry that distinguishes between platelets and small nonbiological particles, eliminating the need for visual recognition and differentiation of debris from platelets.

B.4.2 Platelet aggregation

Platelet aggregation [35] is induced by adding aggregating agents to PRP that is being stirred continually (e.g. ADP, epinephrine, collagen, thrombin, etc.). As the platelets aggregate, the plasma becomes progressively clearer. An optical system (aggregometer) is used to detect the change in light transmission and a recorder graphically displays the variations in light transmission from the baseline setting. Delayed or reduced platelet aggregation may be caused by platelet activation and release of granular contents, increased FDP or certain drugs (e.g. aspirin, nonsteroid anti-inflammatory drugs). It is important to bear in mind that platelet aggregation using some agents varies or may be absent in some animal species. Spontaneous platelet aggregation, occurring in the absence of added agonists, is an abnormal condition indicating activation of platelets.

B.4.3 Blood cell adhesion

Blood cell adhesion [31] is a measure of the blood-compatibility of a material: the fewer blood cells adhere, the more blood-compatible is the surface.

Various methods have been designed to measure the adhesion of cells to surfaces. Most of these methods are based on the observation that a certain proportion of platelets are removed from normal whole blood as a result of passage through a column of glass beads under controlled conditions of flow or pressure. This principle has been adapted to the quantification of the adhesion of other blood cells to polymers coated on glass beads. By such a method it has been reported [31] that adhesion of canine species peripheral lymphocytes and polymorphonuclear leucocytes (PMNs) to beads coated with poly(hydroxyethyl methacrylate) (PHEMA) is lower than to beads coated with polystyrene and certain other polymers. Isolated lymphocytes and PMNs were used in this study.

An alternative method is the direct counting of platelets adherent to a test surface. Following exposure to blood or platelet-rich plasma under standardized conditions, the test surface is rinsed to remove non-adherent cells, fixed and prepared for either light or scanning electron microscopy. The number of adherent platelets per unit area is directly counted and their morphology (e.g. amount of spreading, degree of aggregate formation) is recorded. Alternatively, platelets prelabelled with ^{51}Cr or ^{111}In may be used [30].

B.4.4 Template bleeding time

The commercial availability of a sterile disposable device for producing a skin incision of standard depth and length under standard conditions has significantly improved the reproducibility and value of this test. A prolonged result indicates reduced platelet function or reduced platelet count; the latter can be determined separately (B.4.1). A prolonged bleeding time combined with a normal platelet count has been observed in association with some external communicating devices with limited exposure (e.g. cardiopulmonary bypass) [28]. The test is suitable for use with some experimental animals.

B.4.5 Gamma imaging of radiolabelled platelets

The high gamma emission of ^{111}In enables it to be used for this purpose [20], [27]. This method enables

the localization and quantification of platelets deposited in a device. The technique is useful for external communicating as well as implant devices.

B.4.6 Platelet lifespan (survival)

Platelets are obtained from the patient's blood and are labelled with ^{51}Cr or ^{111}In [20], [21], [32]. Both these agents label platelets of all ages present in the sample, do not elute excessively from the platelets and are not taken up by other cells or reused during thrombopoiesis. Indium-111 has the advantage of being a high gamma emitter, requiring the labelling of fewer platelets and enabling surface body counting to assess localized platelet deposition to be combined with the lifespan study. A reduced platelet lifespan indicates accelerated removal from the circulation by immune, thrombotic or other processes.

B.4.7 PAC-1 and S-12

These are monoclonal antibodies which recognize different epitopes of activated platelets.

B.5 Haematology

B.5.1 Leucocyte count and differential

The use of certain devices may cause leucocyte activation, aggregation and removal from the circulation; a reduced leucocyte count may suggest the existence of such events. An increased leucocyte count may indicate a bacterial infection. A shift in the differential distribution of leucocytes (e.g. granulocytes, lymphocytes, monocytes) may also suggest an infection.

B.5.2 Haemolysis

This is regarded as an especially significant screening test because an elevated plasma haemoglobin level, which if properly performed indicates haemolysis, reflects red blood cell membrane fragility in contact with materials and devices.

B.5.3 Reticulocyte count

An elevated reticulocyte count indicates increased production of red blood cells in the bone marrow. This may be in response to reduced red blood cell mass caused by chronic blood loss (bleeding), haemolysis or other mechanisms.

B.6 Immunology

B.6.1 C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9

Elevated levels of any of these complement components indicate activation of the complement system. Some materials activate complement, and activated complement components in turn activate leucocytes, causing them to aggregate and be sequestered in the lungs.

B.6.2 IL-1 and other cytokines

Elevated levels of some cytokines have been observed following contact of some materials with blood or tissues. Cytokines play a major role in regulating the inflammatory response by controlling the growth of fibroblasts, smooth muscle cells and endothelial cells.

Annex C
(informative)

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Part 10 Methods of tests for dental materials

Part 11 Methods of tests for eye irritation

Part 12 Tests for cytotoxicity: *invitro* methods (*under preparation*)

Part 13 Tests for geno-toxicity, carcinogenecity and reproductive toxicity

Part 14 Selection of tests for interactions with blood

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